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Tobacco-specific nitrosamines in tobacco from U.S. brand and non-U.S. brand cigarettes

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Tobacco-specific nitrosamines (TSNAs) are one of the major classes of carcinogens found in tobacco products. As part of collaborative efforts to reduce tobacco use and resulting disease, the U.S. Centers for Disease Control and Prevention (CDC) carried out a two-phase investigation into the worldwide variation of the levels of TSNAs in cigarette tobacco. In the first phase, representatives of the World Health Organization (WHO) purchased cigarettes; scientists from the CDC subsequently measured the levels of TSNAs in tobacco from 21 different countries. Although the data collected from this initial survey suggested that globally marketed U.S.-brand cigarettes typically had higher TSNA levels than locally popular non-U.S. cigarettes in many countries, the number of samples limited the statistical power of the study. To improve statistical power and to ensure adequate sampling, the CDC conducted a second survey of 14 countries. In addition to the United States, the CDC selected the world's 10 most populous countries and three additional countries, so that at least two countries from each of the six WHO regions were represented. For each country, the CDC compared 15 packs of Marlboro cigarettes, which is the world's most popular brand of cigarettes, with 15 packs of a locally popular non-U.S. brand in the study country. Marlboro cigarettes purchased in 11/13 foreign countries had significantly higher tobacco TSNA levels than the locally popular non-U.S. brands purchased in the same country. The findings suggest that TSNA levels in tobacco can be substantially reduced in some cigarettes.

Introduction

The World Health Organization (WHO) has estimated that there are more than 1.2 billion smokers worldwide, and that annually more than 4 million of these people die from smoking-related disease (Corrao, Guidon, Sharma, & Shokoohi, 2000). The expanding consumption of tobacco products in developing countries and the decreasing consumption in many developed countries such as the United States are shifting the proportion of tobacco-related death and disease toward developing countries (World Bank, 1999). The demand for American-style, blended tobacco products has been projected to grow 3% annually, while cigarette demand grew only 1% a year worldwide ("Global Tobacco," 1996). Philip Morris' Marlboro brand became the world's best-selling cigarette in 1972 and the best-selling brand in the United States in 1975 ("Regal Marlboro," 1996). The Marlboro brand has more than twice the world market share of any of its closest competitors' brands ("Philip Morris," 1993).

Before transnational tobacco companies moved into foreign marketplaces, consumer preferences tended to differ from country to country ("Profile: Philip Morris Europe," 1989). Although some preferences for specific tobacco types still exist around the world, successful marketing strategies and distribution systems and, in some cases, cigarettes with varying nicotine, have resulted in blended tobacco cigarettes becoming the preference in most markets (Hwang, 1998; John, 1989; "Philip Morris European Union," 1996; "Profile: Philip Morris Europe," 1989). The differences in blends, additives, and manufacturing

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processes present an additional challenge in making international comparisons regarding chemical composition of tobacco smoke and the resulting toxicity and carcinogenicity.

Tobacco-specific nitrosamines (TSNAs) comprise a class of known carcinogens that are formed during the curing, processing, fermentation, and combustion of tobacco (Adams, Lee, Vinchkoski, Castonguay, & Hoffmann, 1983; Hoffmann, Brunnemann, Prokopczyk, & Djordjevic, 1994; Spiegelhalder & Bartsch, 1996). TSNAs have been identified in cigarette tobacco (Song & Ashley, 1999; Spiegelhalder & Bartsch, 1996), tobacco smoke (Adams et al., 1983; Djordjevic, Fan, Ferguson, & Hoffmann, 1995), smokeless tobacco (Hoffmann & Adams, 1981; Hoffmann, Adams, Lisk, Fisenne, & Brunnemann, 1987) and other tobacco products such as cigars and bidi cigarettes (Brunnemann, Scott & Hoffmann, 1983; Nair, Pakhale & Bhide, 1989). TSNAs have also been measured in people who smoke and in people exposed to environmental tobacco smoke (Parsons, Carmella, Akerkar, Bonilla & Hecht, 1998; Prokopczyk, Cox, Hoffmann & Waggoner, 1997). Some TSNAs react with DNA (Hecht, 1998) and are carcinogenic in animals. *N*'-nitrosonornicotine (NNN) induces malignant tumors at various respiratory tract sites in mice, rats, mink, and hamsters; 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces lung malignancies in mice, rats, and hamsters (Hecht, 1998; Hoffmann & Hecht, 1985). NNN and NNK, considered to be the most potent of the TSNAs, are believed to be causative agents for human cancers associated with the use of tobacco products (Hecht, 1998, 1999; Hecht & Hoffmann, 1988).

The U.S. Centers for Disease Control and Prevention (CDC) collaborates in worldwide efforts to understand better the relation between tobacco use and disease and to promote tobacco use-prevention. We present the results of a two-part study designed to provide statistically meaningful data on TSNA levels present in a broad survey of international cigarette brands. The purpose of Phase I, which was conducted with help from WHO, was (a) to examine the concentration of a wide variety of chemical compounds in cigarette tobacco and smoke from numerous countries and (b) to help formulate more specific hypotheses about the worldwide variations in tobacco. The results of the initial study suggested further research but were limited statistically because of the relatively low numbers of cigarettes collected from each country. Phase II was a carefully controlled analysis with an improved design based on the results of Phase I. Both phases compare the most widely available multinational brand, Marlboro, with a locally popular non-U.S. brand from each of the participating countries. This study reports results of TSNA analysis in tobacco; a later study will look at levels in tobacco smoke and their relation to tobacco.

Methods

Phase I

In March 1999, we designed a hypothesis-generating study to investigate the differences in chemical composition among commercial cigarettes from around the world and to determine which investigations warranted additional follow-up. Research questions were based on information from tobacco industry documents collected for litigation in Minnesota; those findings suggested significant differences in ventilation and blend recipe design among Marlboro cigarettes marketed internationally (Gordon, 1992). WHO requested that its regional staff and country representatives purchase three packs of Marlboro Full-Flavor cigarettes and three packs of the most popular non-U.S. brand from member countries. The WHO Tobacco-Free Initiative in Geneva, Switzerland, collected the cigarettes and forwarded them to the CDC by mail. Unfortunately, some countries did not follow the collection protocol; Phase I data included only the results from the 21 countries from which sufficient samples were provided. Participating countries in Phase I findings are presented in Table 1.

Phase II

In August 2000, the CDC designed a follow-up study to measure the TSNA content of cigarettes from 14 countries. To increase statistical power, this phase

Table 1. Locally popular non-U.S. brands and WHO regions in the study of tobacco-specific nitrosamines in tobacco from convenience sample (Phase I) cigarettes purchased outside the United States

WHO region	Country	Locally popular non-U.S. brand
AFRO	Botswana	Peter Stuyvesant
AFRO	Kenya	Supermatch
AFRO	Tanzania	Sportsman
EMRO	Jordan	Palace
EMRO	Lebanon	Supars
EMRO	Tunisia	Cristal Extra
EMRO	Yemen	Kamaran
EURO	Armenia	Garni
EURO	France	Gauloises
EURO	Hungary	Sopianae
EURO	Lithuania	Prima
EURO	Romania	Carpati
SEARO	India	St. Express 555
SEARO	Myanmar	London
SEARO	Nepal	Yak
SEARO	Thailand	Krong Thip
WPRO	Laos	A
WPRO	New Zealand	Longbeach
WPRO	Solomon Islands	Benson and Hedges
WPRO	Vanuatu	Peter Jackson
WPRO	Vietnam	Vinataba

AFRO, African Regional Office; EMRO, Eastern Mediterranean Regional Office; EURO, European Regional Office; SEARO, Southeastern Asia Regional Office; WPRO, Western Pacific Regional Office.

involved sampling more cigarette packs in each country than were sampled in Phase I. In addition to the United States (which served as the reference country), the CDC selected the world's 10 most populous countries, plus three other countries, so that at least two countries from each of the six WHO regions were represented. Fifteen packs of Marlboro Full-Flavor cigarettes and 15 packs of a locally popular, full-flavor, non-U.S. brand were purchased by CDC employees who either were stationed or were on temporary duty in each country selected for the study. All cigarettes were purchased between October 2000 and February 2001 on the open market in the countries indicated in Table 2. Marlboro cigarettes purchased outside the United States included those manufactured in the United States and exported or manufactured in facilities outside the United States.

The original collection protocol called for 30 packs of cigarettes to be purchased from each country: five packs of two brands, purchased from the business district or urban area of a major city; five packs of each brand, purchased from a residential or suburban area surrounding a major city; and five packs of each brand, purchased from a rural or farming area near a city. In most countries, this procedure was followed. However, lack of transportation or civil unrest in some countries made the collection protocol impossible. In countries where the protocol could not be followed, packs of cigarettes were collected from diverse locations or at different times. In Japan, only 10 packs of Marlboros were obtained. Using a preprinted label that the CDC furnished and upon which was written a sample identification number, each purchaser labeled the individual packs without breaking the seal or opening the pack. The cigarettes were delivered to the CDC via State Department diplomatic pouch or international overnight carrier, or were delivered by the purchaser. Chain-of-custody sheets accompanied all samples.

Table 2. Locally popular non-US brands and WHO regions in the study of tobacco-specific nitrosamines in tobacco from follow-up sample (Phase II) cigarettes purchased outside the United States

WHO region	Country	Locally popular non-U.S. brand
AFRO	Kenya	Sportsman
AFRO	Nigeria	High Society
AMRO	Brazil	Derby
AMRO	Mexico	Boots
EMRO	Egypt	Cleopatra
EMRO	Pakistan	Embassy
EURO	Germany	West
EURO	Russia	Prima
SEARO	Bangladesh	JP Gold Leaf
SEARO	India	Gold Flake
SEARO	Indonesia	Ardath
WPRO	China	Hongtashan
WPRO	Japan	Mild Seven

AFRO, African Regional Office; AMRO, American Regional Office; EMRO, Eastern Mediterranean Regional Office; EURO, European Regional Office; SEARO, Southeastern Asia Regional Office; WPRO, Western Pacific Regional Office.

Sample handling. After the cigarettes arrived at the CDC, the unopened packs were inspected for integrity, were assigned an identification number, and were logged into a central database system. The samples were then sealed in plastic bags and stored at either −70°C (for storage longer than 2 months) or at 4°C in their original packaging until they were ready to be tested. Prior to analysis, a cigarette from each pack was placed in a chamber set at 60% relative humidity and 22°C for at least 24 hr. Only authorized personnel had access to samples.

For both Phase I and Phase II, we determined the origin of the Marlboro cigarettes by visually inspecting the packs. Those Marlboro cigarettes manufactured in the United States were identified by the words *U.S. tax exempt for use outside U.S.* or *Made in USA* on the pack or on the blue strip where a tax stamp normally resides. The Marlboro cigarettes manufactured outside the United States listed the location of manufacture and/or had one of the following designations: *Made under license from Philip Morris*, *Made under authority of Philip Morris*, or *Trademark owner Philip Morris*.

TSNA analysis. The extraction and isotope dilution gas chromatography–mass spectrometry (GC-MS) analysis method for TSNAs is a modification of a previous method described elsewhere (Song & Ashley, 1999); the method involves using automated solvent extraction (ASE) instead of supercritical fluid extraction. Approximately 0.5 g of tobacco was weighed, spiked with 10 μg of $^{13}C_6$-labelled NNK as internal standard, and then extracted with ethyl acetate using the Dionex ASE 200 (Sunnyvale, CA) accelerated solvent extractor. The extract was washed twice with 6 N NaOH to remove interferences and then further purified using Waters (Milford, MA) OASIS HLB solid phase extraction columns. The eluent was then analyzed using an Agilent (Palo Alto, CA) 6890/5973 GC-MS. For both NNN and NNK, two fragment ions were monitored. One mass was monitored for quantifying the amount of NNN or NNK present in the sample. The second mass was monitored for confirmation purposes; the ratio of the area of the first mass to the area of the second mass was required to be within predetermined limits to verify the analyte identity. Tobacco from the filtered research cigarette 1R4F (University of Kentucky, Louisville, KY) was used as a quality control material and was included with every analytical run. Linear regression analysis of standard concentration versus relative peak area was used to relate peak areas to analyte

concentration. Total carcinogenic TSNA levels were determined by summing the levels of NNN and NNK per gram wet weight of tobacco.

Statistical analyses. Statistical analyses were performed using SAS statistical software (SAS Institute, Inc., Cary, NC). Within each country, total carcinogenic TSNAs in Marlboro and locally popular non-U.S. brands were compared using *t*-tests after evaluation of the equality of distribution variances. Differences were considered statistically significant when the p value was less than 0.05 for a two-sided *t*-test.

Results

Phase I

Figure 1 shows a comparison of total carcinogenic TSNA levels (NNN + NNK) measured in the tobacco from 21 countries in Phase I. Statistical comparison of the U.S. brand (Marlboro) and locally popular non-U.S. brand for each country was of limited utility because of the small number of samples available from each country. In 15 (71%) of the 21 countries shown, the Marlboro brand

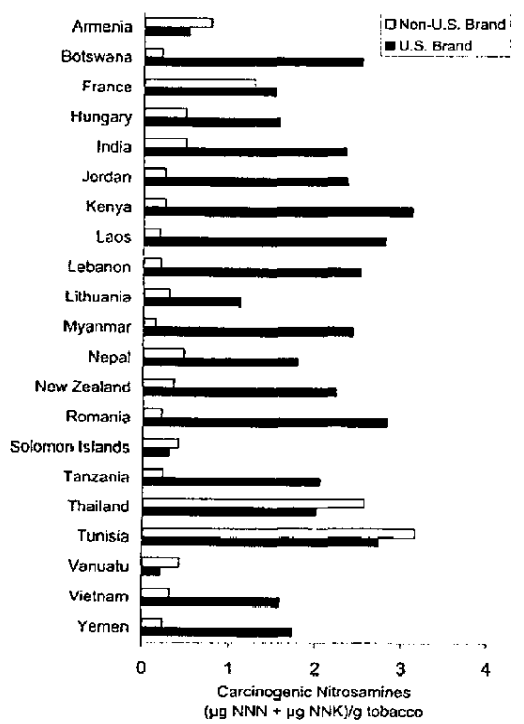

Figure 1. Amounts of *N'*-nitrosonornicotine (NNN) + 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in µg/g measured in the Phase I cigarettes. The closed bars represent the amount measured in Marlboro. The open bars represent the amount measured in the locally popular non-US brand.

appeared to contain substantially more TSNAs than the locally popular non-U.S. brand purchased in the same country. In one other country (France), TSNA levels in the Marlboro tobacco brand were higher than the locally popular non-U.S. brand, but not substantially so. In two of the countries (Thailand and Tunisia), the TSNA levels in both brands of cigarettes were higher than 2 µg/g tobacco, with levels of the locally popular non-U.S. brand being higher than those of the Marlboro. In the three remaining countries (Armenia, Solomon Islands, and Vanuatu), the TSNAs in tobacco from both the Marlboro and the locally popular non-U.S. brand were relatively low, with TSNA levels in the locally popular non-U.S. brand being somewhat higher. In addition, levels of TSNAs in tobacco from Marlboro cigarettes varied more than 10-fold among the samples examined.

We conducted this same analysis using supercritical fluid extraction (SFE), which is a more extensive extraction process, on 16 of the samples that had been analyzed using ASE. Table 3 shows the comparison of the levels using both extraction methods. The findings using this alternate extraction technique were similar, although the actual levels of TSNAs in the tobacco were higher and the differences were more pronounced. Again, for most of the countries examined, the tobacco from the Marlboro cigarette, extracted using SFE, had substantially higher levels of TSNAs than the tobacco from the locally popular non-U.S. brand. The results using both of these techniques suggested the need for a more statistically rigorous study.

Phase II

Total TSNA levels (NNN + NNK) measured in tobacco from cigarettes purchased during Phase II are shown in Table 4. The mean with the standard error of the mean is shown for each country as well as the median and the number of packs investigated per brand. The p value represents the comparison of the means of the Marlboro and the locally popular non-U.S. brand. The means for total TSNA levels in the tobacco from these cigarettes vary widely, with the lowest value being 0.087 µg/g and the highest value being 1.9 µg/g, reflecting a more than 20-fold difference. In 11 (85%) of the 13 non-U.S. countries investigated, the mean TSNA levels in Marlboro tobacco were significantly higher than mean TSNA levels in the non-U.S. brand, regardless of where the Marlboro was manufactured. In cigarettes purchased in Mexico, the means of total TSNA levels in Marlboro and in the non-U.S. brand were not statistically significant. In cigarettes purchased in Brazil, the mean TSNA level from the non-U.S. brand was significantly greater than the mean TSNA level in Marlboros. The comparison brand purchased in the United States, Doral (the second most popular non-menthol brand sold in the United States), is

Table 3. Sum of *N'*-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in cigarette tobacco from locally popular and Marlboro cigarettes purchased outside the United States using two different extraction methods in Phase I

Country	Locally popular non-U.S. brand	Carcinogenic TSNAs in locally popular cigarettes		Carcinogenic TSNAs in Marlboro cigarettes	
		ASE extraction	SFE extraction	ASE extraction	SFE extraction
Armenia	Garni	.772	.671	.52	.56
Botswana	Stuyvesant	.209	.208	2.52	5.47
France	Gaulcises	1.26	1.18	1.35	3.81
Hungary	Sopianae	.487	.343	1.56	2.88
Jordan	Palace	.250	.313	2.36	5.87
Laos	A	.190	.580	2.80	5.48
Lebanon	Supars	.206	.346	2.52	6.87
Lithuania	Prima	.297	.541	1.11	3.70
Myanmar	London	.145	.632	2.43	6.58
Nepal	Yak	.467	.622	1.78	5.39
New Zealand	Longbeach	.353	1.02	2.23	6.96
Romania	Carpati	.218	.839	2.82	5.71
Solomon Islands	Benson and Hedges	.411	1.22	.31	1.62
Vanuatu	Peter Jackson	.431	.720	.21	1.31
Vietnam	Vinataba	.324	.626	1.59	4.76
Yemen	Kamaran	.237	.382	1.73	4.98

ASE, accelerated solvent extraction; SFE, supercritical fluid extraction

Table 4. Mean, standard error of the mean, median, and *t*-test *p* value for sum of *N'*-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in cigarette tobacco from locally popular and Marlboro cigarettes purchased in Phase II

Country	Brand	Number of packs	Mean (μg/g) ± standard error of the mean	Median (μg/g)	*p* value
Bangladesh	JP Gold Leaf	15	.22 ± .016	.23	<.0001
	Marlboro	13	1.9 ± .14	1.9	
Brazil	Derby	15	1.1 ± .067	1.1	.005
	Marlboro	14	.74 ± .10	.62	
China	Hongtashan	15	.087 ± .008	.088	<.0001
	Marlboro	15	1.9 ± .146	1.9	
Egypt	Cleopatra	15	.67 ± .082	.60	<.0001
	Marlboro	13	1.5 ± .10	1.4	
Germany	West	14	.58 ± .041	.61	<.0001
	Marlboro	13	1.2 ± .066	1.1	
India	Gold Flake	15	.10 ± .010	.093	.0001
	Marlboro	15	.90 ± .15	.66	
Indonesia	Ardath	15	.44 ± .034	.41	<.0001
	Marlboro	13	1.9 ± .18	1.9	
Japan	Mild Seven	14	.47 ± .049	.42	.004
	Marlboro	10	.93 ± .12	.83	
Kenya	Sportsman	15	.16 ± .018	.16	<.0001
	Marlboro	15	1.8 ± .20	1.5	
Mexico	Boots	15	1.1 ± .11	.92	.65
	Marlboro	15	1.0 ± .095	1.0	
Nigeria	High Society	15	.22 ± .008	.21	.005
	Marlboro	15	1.0 ± .23	.36	
Pakistan	Embassy	15	.29 ± .024	.30	<.0001
	Marlboro	12	1.5 ± .094	1.4	
Russia	Prima	15	.94 ± .074	.87	.03
	Marlboro	11	1.2 ± .11	1.1	
USA	Doral	14	1.5 ± .085	1.5	.93
	Marlboro	15	1.5 ± .085	1.6	

produced by a different U.S. manufacturer. The levels of TSNAs in tobacco from Doral and the U.S.-purchased Marlboro were not significantly different.

The analysis of TSNA levels in tobacco from most of the brands analyzed in Phase II had coefficients of variation (CVs) between 15% and 40%; India Marlboro had a CV of 66% and Nigeria Marlboro had a CV of 90%. The inclusion of cigarettes from multiple packs purchased at different times and, in many cases from different locations, incorporates the natural variability related to the manufacturing process and to shelf life, along with the analytical variation in the measurement. Five cigarettes from the same pack were examined to estimate the intrapack

variability. The CV from this analysis was 24%. The interpack variability, determined on 15 cigarettes from 15 packs of the same brand, was 22%. For 12/14 countries sampled in this study, the CVs for tobacco TSNAs were in this same approximate range, with India and Nigeria having much higher CVs.

Discussion

Phase I was intended primarily to be a hypothesis-generating study to identify differences in tobacco constituents of public health concern and to determine whether further investigations were warranted. In 15 of the 21 (71%) non-U.S. countries that contributed sufficient Marlboro and locally popular non-U.S. brand cigarettes for comparison, TSNA levels in Marlboro cigarettes were higher than levels in the non-U.S. brand. Overall, the results of Phase I indicated that TSNA levels in cigarette tobacco differed significantly from country to country and often differed between Marlboros and non-U.S. brands.

In 10/13 non-US countries examined in Phase II, the mean level of TSNAs in Marlboro cigarettes was at least two times the mean level of TSNAs in tobacco from non-U.S. brands. In Mexico and Brazil, the non-U.S. brands had higher TSNA levels than Marlboros; the difference in Mexico was not statistically significant. In Russia, the TSNA levels in Marlboros were 1.3 times the level in the non-U.S. brand. In Egypt, Germany, and Japan, the TSNA levels in the Marlboros were approximately two times the levels in the non-U.S. brands. In the remaining countries, the Marlboro TSNA levels were much higher than the non-U.S. brands, ranging between 4.4 and 22 times higher. This finding was particularly notable in China, where approximately 30% of the world's cigarettes are manufactured and consumed.

Whereas TSNA levels in cigarettes and cigarette smoke have been reported to vary both within and among countries, most studies examined cigarettes from a limited geographical region. For example, reports of TSNA levels in cigarettes obtained from several European countries and the United States (Fischer, Spiegelhalder, & Preussmann, 1990a; Spiegelhalder & Bartsch, 1996) indicated that TSNA levels in cigarette tobacco varied widely within a single country, although the authors did not note any significant differences between countries. Fischer, Spiegelhalder, and Preussmann (1989b) analyzed TSNA levels in smoke from 55 brands of West German cigarettes. The researchers found a significant range of TSNA levels in smoke but determined that "tar" delivery was not a sufficient index for the carcinogenic potential of smoke. Kowalski (1995) compared TSNA levels in tobacco from Polish cigarettes with TSNA levels in tobacco from German, Indian, and Canadian cigarettes. TSNA levels in Polish cigarettes were significantly higher than levels in cigarettes from the other countries. In a limited report, Gray, Boyle, and Zatonski (1998) reported that TSNA levels in cigarette tobacco varied substantially. Atawodi, Preussmann, and Spiegelhalder (1995) examined TSNAs in tobacco and tobacco smoke from cigarettes purchased in Nigeria; they did not find a significant difference in TSNAs when they compared the Nigerian cigarettes with European and American cigarettes. A study of TSNAs in tobacco and smoke from Canadian cigarettes (Fischer, Castonguay, Kaiserman, Spiegelhalder, & Preussman, 1990b) indicated that when cigarettes are made from a single type of tobacco, "tar" delivery is a good predictor of TSNA levels in smoke. They attributed this finding primarily to the relation between "tar" delivery and ventilation. Finally, an international group of researchers (Gray et al., 2000) found significant variation in the TSNA levels of three multinational brands purchased in 29 countries. While significant numbers of cigarettes were sampled to provide valid statistics, not all brands were available in each country. Based largely on the variations in TSNA levels, Gray and colleagues concluded that tobacco companies have the ability to produce cigarettes with lower TSNA levels. The results reported in this manuscript also show a wide variation in tobacco TSNA levels, even from the same brand. In a recent study, Stepanov, Carmella, Hecht, & Duca (2002), compared tobacco TSNAs from Moldovan and "American-type" cigarettes. In agreement with the findings of the data reported here, tobacco TSNAs in American-brand cigarettes were significantly higher than in Moldovan cigarettes.

In a previous report (Song & Ashley, 1999), we compared the analytical results from mass spectrometry and thermal energy analysis (TEA), the most commonly used analytical technique for TSNA measurement, and found that the levels of TSNAs determined by these two methods were equivalent. We have repeated many of the analyses reported in this manuscript using TEA. The results using this instrumentation showed differences in tobacco TSNA levels between the non-U.S. brand and Marlboro that were similar to those reported in Table 4. Compared with TEA, mass spectrometric detection provides additional method selectivity by ensuring the identity of the analyte being measured.

The aroma of cigarette tobacco results from volatile natural constituents and additives. These volatile components can be analyzed to provide a useful tool in evaluating one fraction of the chemical species in cigarette tobacco. We applied this technique in a previous investigation (CDC, 1996). In a separate study, we examined the levels of volatile organic compounds (VOCs) in tobacco isolated from the same cigarette samples for which TSNA levels were

determined. Specific VOC constituents are present in tobacco from Marlboro cigarettes. VOC levels in the samples examined in this study suggested that the 15 samples of Marlboro cigarettes purchased in Nigeria should be divided into two separate groups—one that did not correspond to the typical Marlboro VOC pattern and one that did. The first of these groups, consisting of nine of the packs purchased, had low TSNA levels (approximately 0.3 μg/g). The second group, which consisted of the remaining six packs and yielded the typical Marlboro VOC pattern, contained tobacco with a significantly higher level of TSNAs (approximately 2.0 μg/g). This level is similar to many of the other Marlboro cigarettes purchased during this study. It is unlikely that Marlboro cigarettes purchased in Nigeria have significant chemical variability, because of the efforts of Philip Morris to keep their product consistent ("Profile: Philip Morris Europe," 1989). It also is possible that these samples were counterfeit cigarettes, since the counterfeiting of well-recognized U.S. brands is a common problem worldwide (Dawn, 2001; Lewis, 2000; Philip Morris, 2001). Similar results were found for the Marlboro cigarettes purchased in India, resulting in the wide range of measured TSNA levels in tobacco from this country.

Table 5 presents the mean TSNA measurements in Marlboro cigarette tobacco by WHO region from Phase II. With the exclusion of variability that may be related to counterfeiting, the mean TSNA levels in Marlboro cigarettes from each WHO region are similar, except for the Western Pacific Regional Office (WPRO). However, more substantial differences in means exist among WHO regions. Mean TSNA levels in tobacco from Nigeria and India correspond more closely to those of the other countries in their region when the samples with an atypical VOC constituent pattern are excluded. TSNAs in tobacco from Marlboro cigarettes purchased in Japan are significantly lower than TSNAs in tobacco from Marlboro cigarettes purchased in China. This finding may have occurred because Marlboro-labeled cigarettes for sale in Japan are actually manufactured by Japan Tobacco. When such exceptions are taken into account, regional differences in Marlboro TSNA levels are clarified. Tobacco from Marlboro cigarettes from WPRO, the Southeastern Asia Regional Office, and the African Regional Office had the highest levels of TSNAs, followed by the Eastern Mediterranean Regional Office, the European Regional Office, and the American Regional Office. Previously, trade journals have discussed Philip Morris' effort to maintain a consistency in their product geographically ("Profile: Philip Morris Europe," 1989). This effort results in a consistent smoke taste among tobacco from different countries. Our findings of regional consistency for Marlboro cigarettes suggest that this consistency extends to levels of TSNAs across countries within close geographical proximity. Tobacco TSNA levels in Marlboro cigarettes manufactured within the United States for export and those manufactured outside of the United States did not differ statistically.

Table 5. Carcinogenic tobacco-specific nitrosamines in tobacco from Marlboro cigarettes purchased outside the United States; listed by WHO region

WHO region	Country	Manufactured in U.S.	Mean TSNA level (μg/g)
AFRO	Kenya	Yes	1.8
AFRO	Nigeria[a]	Yes	1.0
AMRO	Brazil	No	.74
AMRO	Mexico	No	1.0
EMRO	Egypt	No	1.5
EMRO	Pakistan	Yes	1.5
EURO	Germany	No	1.2
EURO	Russia	No	1.2
SEARO	Bangladesh	No	1.9
SEARO	India[a]	Yes	.90
SEARO	Indonesia	No	1.9
WPRO	China	Yes	1.9
WPRO	Japan[b]	No	.93
	USA		1.5

AFRO, African Regional Office; AMRO, American Regional Office; EMRO, Eastern Mediterranean Regional Office; EURO, European Regional Office; SEARO, Southeastern Asia Regional Office; WPRO, Western Pacific Regional Office.

[a]Further chemical analysis of these samples suggested two separate cigarette types (India: means ± standard deviation, 1.64 ± .40 and .537 ± .20; Nigeria: means, 2.03 ± .36 and .307 ± .043).

[b]In Japan, Philip Morris has licensed the manufacture of Marlboro to Japan Tobacco.

The typical U.S.-blended cigarette contains a mix of bright, burley, Oriental, reconstituted, and expanded tobaccos (Hoffmann & Hoffmann, 1997). The types of tobacco used throughout the world to manufacture cigarettes differ substantially depending on cost, taste preferences, government regulations, and other considerations. In some countries, cigarettes primarily contain a single tobacco type (e.g., bright tobacco in the U.K.). The different types of tobacco used to manufacture cigarettes contain different levels of TSNAs, and the blending of these tobaccos can have a significant effect on the levels of nitrosamines in the tobacco and subsequently in the smoke (Fischer, Spiegelhalder, & Preussmann, 1989a, b; Hoffmann & Hoffmann, 1997; Spiegelhalder & Bartsch, 1996).

Levels of TSNAs also can vary within batches of flue-cured tobacco. The level of TSNAs in tobacco is affected by the amount of nitrate present during growing and curing (Hoffmann & Hoffmann, 1997; Chamberlain, Baker, Chortyk, & Stephenson, 1986). The use of direct-fire burners fueled by propane gas to flue-cure tobaccos began in the late 1960s and early 1970s (Fisher, 2000). These burners exhaust combustion gases directly into the tobacco-curing barn, exposing the curing tobacco to NO_x gases that result from incomplete fuel combustion. These gases react with alkaloids in the tobacco to form TSNAs. Tobacco curing operations that do not expose the

curing tobacco to exhaust gases (e.g., heat exchange curing methods) eliminate this source of TSNA formation. TSNAs also are found in burley tobacco; researchers believe that those TSNAs result from microbial reduction of nitrate to nitrite and other NO_x compounds. Different blending and curing practices most likely are responsible for the variation in TSNA levels reported here.

The factors that influence TSNA formation during curing and burning are similar. It has been shown that with smoking machine-generated smoke, TSNA levels in smoke depend on TSNA levels in tobacco when equivalent ventilation and smoking parameters are used (Fischer et al., 1990a; Spiegelhalder & Bartsch, 1996). For cigarettes constructed of similar tobacco types, TSNA levels in smoke also depend on dilution of the smoke stream by air pulled through the paper and ventilation holes in the cigarette (Fischer et al., 1990b). Smoking behavior also influences the amount of TSNAs that a smoker receives (Djordjevic et al., 1995). Thus, when comparing cigarettes with the same level of ventilation, the level of TSNAs in tobacco correlates with the extent to which the smoker is exposed to TSNAs, as long as a person's smoking behavior does not change (Fisher, Spiegelhalder, Eisenbarth, & Preussmann, 1990c).

The "tar" level determined using standardized automated smoking-machine parameters was once considered a good index of the carcinogenic potential of mainstream smoke (International Agency for Research on Cancer [IARC], 1986). In more recent years, experimental evidence has shown that, when the wide variety of cigarettes available worldwide are examined, the "tar" delivery measured using the Federal Trade Commission method is not correlated with the levels of NNN and NNK in smoke (Fischer et al., 1989b, 1990a; Spiegelhalder & Bartsch, 1996). The significant differences in the chemical composition of "tar" result from the burning of different tobacco types. Thus, both the chemical composition and the amount of "tar" delivered must be evaluated when considering the carcinogenic potential of mainstream smoke.

A U.S. Surgeon General's report (CDC, 2000) stated, "As with other consumer products, the manufactured tobacco product should be no more harmful than necessary given available technology." Mainstream and sidestream tobacco smoke contain at least five primary classes of carcinogenic substances. These include VOCs (e.g., benzene and formaldehyde), heavy metals (e.g., cadmium, nickel, and polonium-210), aromatic amines (e.g., 2-toluidine and 4-aminobiphenyl), polyaromatic hydrocarbons (e.g., benzo[a]pyrene and benz[a]anthracene), and nitrosamines (e.g., NNN and NNK) (Hoffmann & Hoffmann, 1997, 1998; IARC, 1986). We examined two chemicals from one of these five groups—the TSNAs, NNN and NNK. Both of these tobacco constituents are classified as possibly carcinogenic to humans (Group 2B) by the IARC (1985). Researchers have hypothesized that lowering the levels of harmful components of tobacco and tobacco smoke could lead to reduced exposure to carcinogens and reduced incidence of disease resulting from tobacco use (Adams et al., 1983; Atawodi et al., 1995). Although the present study did not address this possibility, results indicate that different brands of cigarettes contain differing amounts of carcinogenic TSNAs, indicating that it is possible for manufacturers to produce cigarettes with lower levels of these toxic substances.

TSNAs show the highest concentration of any group of strong carcinogens in mainstream cigarette smoke (Hecht & Hoffmann, 1988). However, TSNAs are not the only carcinogens found in cigarette smoke, and reducing their levels alone does not guarantee a less hazardous cigarette. All sources of risk and factors altering risk must be considered in evaluations of the relative risk of cigarette smoke, including the concentration of all of the harmful smoke constituents as well as the potency and biological availability of each component.

The most effective ways to minimize tobacco-attributable health risks are not starting tobacco use, quitting tobacco use completely, and avoiding exposure to environmental tobacco smoke. The CDC continues to work with WHO's Tobacco-Free Initiative to enhance efforts to prevent and reduce tobacco use worldwide. However, manufacturers should follow the advice of the U.S. Surgeon General to manufacture cigarettes that are not more harmful than necessary, given available technology, including reducing the levels of TSNAs and other toxic substances in cigarettes.

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